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Dear Editorial Board at *Nature*,

Atmosphere-biosphere carbon flux predictions diverge across land surface models when simulated using future climatic scenarios. Studies show that these models are particularly sensitive to the simulation of photosynthetic processes in response to increasing temperature and CO2 concentration1,2, yet few models incorporate explicit frameworks for simulating plant acclimation responses to such changes3.

Plants acclimate to increasing CO2 concentrations by reducing leaf nitrogen (N) content and photosynthetic capacity, a pattern that often corresponds with increased operational net photosynthesis rates and acute increases in total leaf area and biomass accumulation. Progressive N limitation has been hypothesized to be the primary mechanism driving increased growth rates under elevated CO2, as N availability commonly limits primary productivity4. The progressive N limitation hypothesis predicts that elevated CO2 increases plant nitrogen demand to build and maintain photosynthetic tissue, which increases plant nitrogen uptake rates and causes soil nitrogen availability to progressively decline over time. The hypothesis predicts that declines in soil nitrogen availability over time will decrease leaf N and photosynthetic capacity over time and that acute increases in total leaf area and biomass will dampen over time as nitrogen availability becomes progressively more limited. However, progressive nitrogen limitation does not explain why newly expanded leaves often have increased operational net photosynthesis rates despite reduced photosynthetic capacity, and limited empirical evidence exists supporting such an integrated role of soil nitrogen availability on concurrent leaf and whole plant responses to elevated CO25.

An alternative hypothesis to explain plant responses to elevated CO2 suggests that the reduction in leaf N and photosynthetic capacity is the result of an allocation strategy that allows plants to optimize resource use efficiency at the leaf level, maximizing relative N allocation to photosynthetic enzymes and structures supporting whole plant growth. Optimal resource allocation to photosynthetic capacity uses principles from optimal coordination theory to suggest that plants optimize leaf photosynthetic processes by optimally allocating N to Rubisco carboxylation such that net photosynthesis rates are equally co-limited by the maximum rates of Rubisco carboxylation and RuBP regeneration6,7. If true, the theory predicts that plants should optimally respond to elevated CO2 by decreasing leaf nitrogen content and increasing relative N investment to photosynthetic enzymes, leading to an increase in operational net photosynthesis rates at reduced photosynthetic capacity while maximizing N allocation to structures supporting whole plant growth. The expected optimal leaf response to elevated CO2 has received some empirical support8, though no studies have connected these patterns with concurrent leaf and whole plant responses to elevated CO2. Importantly, this theory suggests that leaf responses to elevated CO2 are independent of soil N availability, though does not discount potential positive roles of soil N availability on whole plant growth responses to elevated CO2.

To disentangle effects of soil N availability on leaf and whole plant responses to elevated CO2, we grew *Glycine max* under one of two CO2 concentrations (420 ppm and 1000 ppm), one of two inoculation treatments (inoculated and uninoculated with *Bradyrhizobium japonicum*), and one of nine soil N fertilization treatments in a full-factorial growth chamber experiment. After seven weeks of vegetative growth, we measured leaf N content, conducted net photosynthesis-by-intercellular CO2 concentration curves to estimate the maximum rates of Rubisco carboxylation and RuBP regeneration, and destructively harvested individuals to quantify total leaf area and whole plant biomass. We also calculated the fraction of leaf N allocated to photosynthetic and structural tissue9 and structural carbon costs to acquire nitrogen10.

We find that elevated CO2 decreased leaf nitrogen content, the maximum rate of Rubisco carboxylation, and the maximum rate of RuBP regeneration. Elevated CO2 decreased the maximum rate of Rubisco carboxylation more strongly than the maximum rate of RuBP regeneration and increased the relative fraction of leaf N content allocated to photosynthetic tissue, allowing leaves to approach optimal co-limitation of Rubisco carboxylation and RuBP regeneration rates by optimizing nutrient allocation to photosynthetic enzymes. In all cases, leaf responses to CO2 were independent of fertilization or inoculation treatment, negating patterns expected from progressive nitrogen limitation. Interestingly, elevated CO2 increased total leaf area and total biomass, responses that were enhanced with increasing fertilization and associated with reductions in the cost of acquiring nitrogen and increases in nitrogen uptake.

Our results provide support for both the N limitation and optimal coordination hypotheses, though suggest that each hypothesis operates on a different scale. Specifically, results from this experiment indicate that leaf acclimation responses to elevated CO2 were indicative of patterns expected from optimal resource allocation to photosynthetic capacity, while whole plant responses to elevated CO2 were indicative of patterns expected from progressive nitrogen limitation.

Elevated CO2 experiments rarely quantify both leaf and whole plant responses concurrently within the same experiment, and studies linking these responses often rely on meta-analyses from different studies11,12. Our findings integrate both leaf and whole plant responses to elevated CO2 and relate these findings to plant nitrogen uptake rates and costs of nitrogen acquisition. Therefore, we feel this paper provides a novel, potentially paradigm-shifting contribution to the plant ecophysiological and modelling communities, and expect this paper to be cited broadly in both communities. Given this, I submit this letter on behalf of my coauthors as pre-submission inquiry to *Nature* and the associated *Nature* family of journals. Attached to this cover letter is the initial summary paragraph of the proposed *Nature* article.

Sincerely,

Evan A. Perkowski

*On behalf of coauthors Ezinwanne Ezekannagha and Nicholas G. Smith*

**Summary paragraph**

Plants respond to elevated CO2 concentrations by reducing leaf nitrogen allocation and photosynthetic capacity, an acclimation response that coincides with increased growth rates and total leaf area over short time scales that dampen with time11,13. Progressive nitrogen limitation has been hypothesized to be the primary mechanism driving leaf and whole plant responses to elevated CO2, as nitrogen availability limits net primary productivity globally4,14 due to high nitrogen requirements to build and maintain photosynthetic enzymes15,16. Recent work calls this hypothesis into question, suggesting that leaf responses to elevated CO2 are independent of nitrogen availability and are instead the result of optimal resource investment to photosynthetic capacity. Despite empirical support for both hypotheses5,8,17, studies that quantify leaf and whole plant responses to elevated CO2 generally assess such responses using meta-analytic techniques 11,12, and studies that examine leaf and whole plant responses concurrently are rare. Here, we show that reductions in photosynthetic capacity under elevated CO2 were associated with increased fractions of leaf nitrogen content allocated to photosynthetic capacity, patterns that were each independent of soil nitrogen fertilization. We also show that increased whole plant growth and total leaf area under elevated CO2 were enhanced with increasing fertilization, a pattern that was associated with increased plant nitrogen uptake rates with increasing fertilization. Results from this experiment resolve discrepancies between optimal resource allocation and progressive nitrogen limitation, showing that optimal resource allocation to photosynthetic capacity drives leaf acclimation responses to elevated CO2, while patterns expected from progressive nitrogen limitation in turn drive whole plant responses to elevated CO2. The differential role of soil nitrogen availability on leaf and whole plant responses to elevated CO2 build on previous work suggesting that land surface models may improve their simulation of photosynthetic processes under future novel environments by adopting frameworks that include optimality principles8,18,19.

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